Advanced Use of NCBI Resources David Wheeler, Ph.D. National Center for Biotechnology Information National Institutes of Health

Genomics and Proteomics in Kidney and Urologic Diseases

July 10, 2001

Advanced Use of NCBI Resources Start Here:

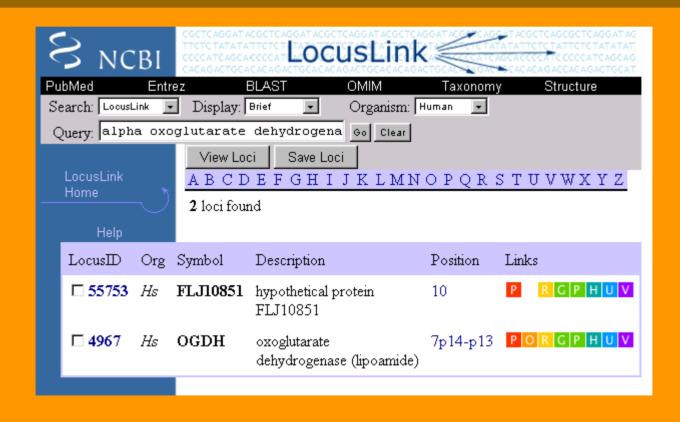
http://www.ncbi.nlm.nih.gov/

Finding a New Gene
Finding a 3D Structural Model
Genomic Comparisons
Sifting Through Shotgun Sequence

Searching for an Unannotated Gene within the Human Genome

Evaluation of the Implied Protein Sequence

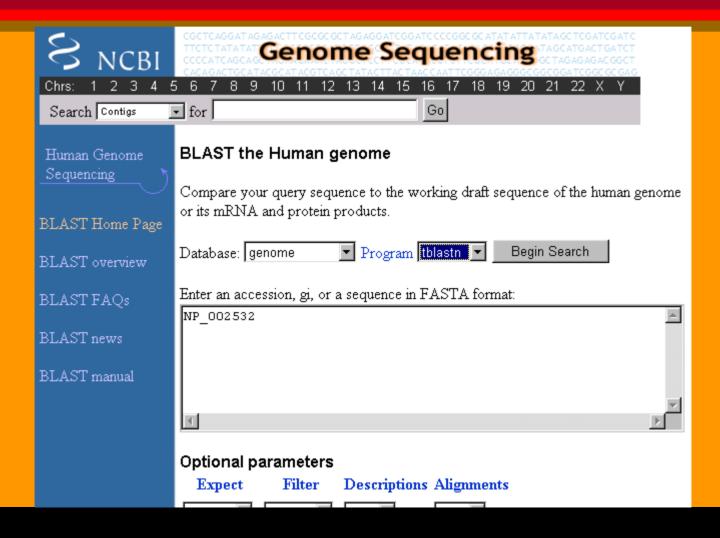
Using LocusLink to Find a Probe Sequence



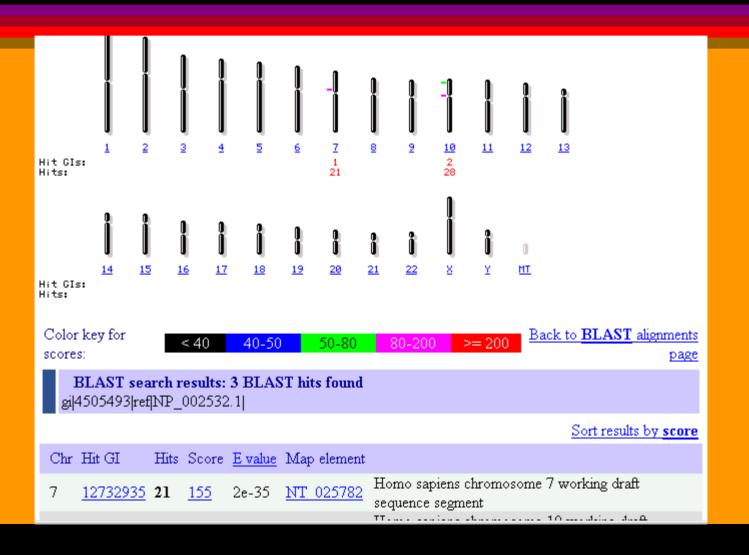
The Protein RefSeq is the more Sensitive Probe

LocusLink Home											
	NCBI Reference		?								
OGDH Index:	Category: PROVISIONAL										
Top of Page Nomenclature	mRNA: <u>NM 002541</u>										
Overview	Protein: NP 002532 oxoglutarate dehydrogenase										
Function	(lipoamide)										
Relationships	Domains:	score: 792									
Map .	GenBank Source: D10523										
RefSeq	300000 <u>210302</u>										
GenBank	Category: NCBI Genome Annotation										
Links	Genomic Contig: NT 025782 sv mv										
LocusLink	Evidence: supported by alignment with										
Collaborators	mRNA										
Download	Model mRNA: XM 004889										
FAQ	Model Protein:				BL						
Help	Domains:		e E1 component	score: 752							
Statistics	Domants.	<u>Denyar ogenas</u>	<u>c Dr component</u>	30010. 752							
DofCog:	GenBank Sequen	ices			?						
RefSeq:	Nucleotide	Туре	Protein								
Download	D32064	g	BAA06836		BL						
FAQ	BC004964	m	AAH04964		BL						
Statistics	D10523	m	BAA01393		BL						
	1710323	111	<u> </u>		J_						

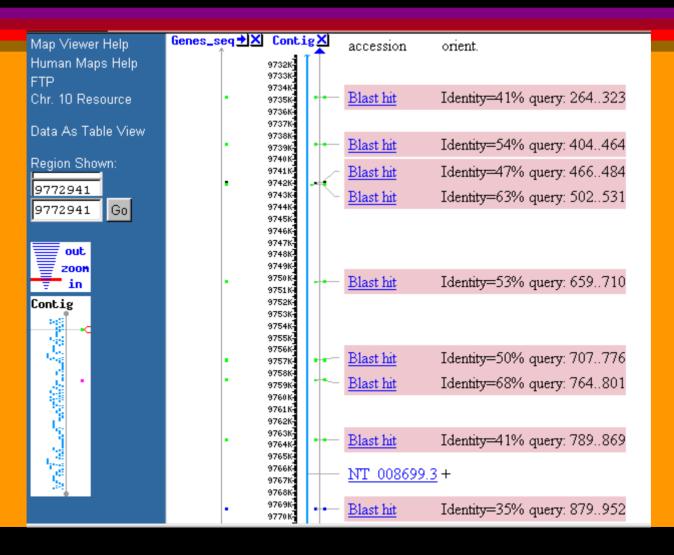
A tblastn Search using Human Genome BLAST



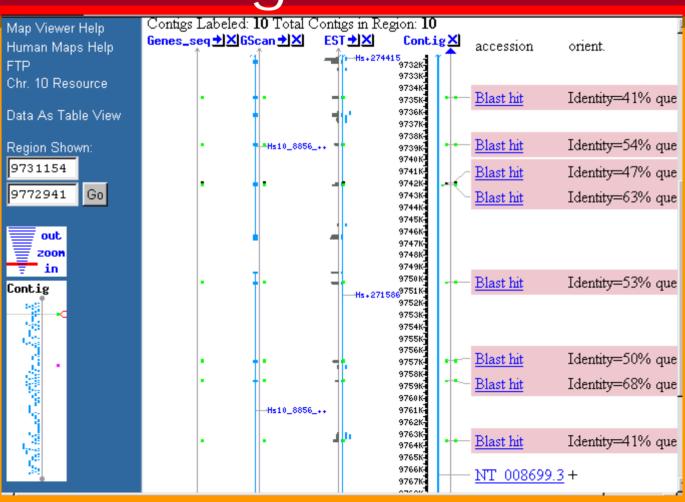
Three Hits



BLAST HIts but no RefSeq Alignments



GenomeScan Gene Models and EST Alignments



Some Information From Unigene Cluster 271586

Home Page

Frequently Asked Questions

Query Tips

Library Differential Display

Download UniGene

JniGene Homo sapiens

Home Page

Release Statistics

Library Report

Library Browser

SEE ALSO

LocusLink: 55526

HomoloGene: Hs.271586

SELECTED MODEL ORGANISM PROTEIN SIMILARITIES organism, protein and percent identity and length of aligned region

H.sapiens: pir:T50617 - T50617 hypothetical protein 100 % / 539 aa

DKFZp762M115.1

M.musculus: prf.2001488A - 2-OXOGLUTARATE 48 % / 128 aa

DEHYDROGENASE E1 COMPONENT

C.elegans: pir:T28034 - T28034 hypothetical protein 50 % / 897 aa

ZK836.2 - Caenorhabditis elegans

S.cerevisiae: sp:P20967 - ODO1 YEAST 38 % / 859 aa

2-OXOGLUTARATE DEHYDROGENASE E1

COMPONENT, MITOCHONDRIAL

PRECURSOR (ALPHA-KETOGLUTARATE D

E.coli: pir:DEECOG - DEECOG oxoglutarate 40 % / 856 aa

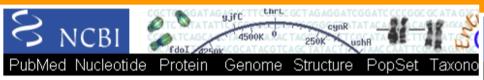
dehydrogenase (lipoamide) (EC 1.2.4.2) -

Escherichia coli

EXPRESSION INFORMATION

cDNA sources: Brain, Breast, Esophagus, Head and neck, Kidney, Tonsil,

Two GenomeScan Predicted Peptides



PubMed Nucleotide Protein Genome Structure PopSet Taxono

Homo sapiens Map View

GenomeScan model Hs10_8856_22_1_1

CDS:

>lc1|Hs10_8856_22_1_1

MASATAAAAARRGLGRALPLLWRGYQTERGVYGYRPRKPESREPQGALERPPVDHGLARLV
TVYCEHGHKAAKINPLFTGQALLENVPEIQALVQTLQGPFHTAGLLNMGKEEASLEEVLV
YLNQIYCGQISIETSQLQSQDEKDWFAKRFEELQKETFTTEERKHLSKLMLESQEFDHFL
ATKFSTVKRYGGEGAESMMGFFHELLKMSAYSGITDVIIGMPHRGRLNLLTGLLQFPPEL
MFRKMRGLSEFPENFSATGDVLSHLTSSVDLYFGAHHPLHVTMLPNPSHLEAVNPVAVGK
TRGRQQSRQDGDYSPDNSAQPGDRVICLQVHGDASFCGQGIVPETFTLSNLPHFRIGGSV
HLIVNNQLGYTTPAERGRSSLYCSDIGKLVGCAIIHVNGDSPEEVWGHNELDEPFYTNPI
MYKIIRARKSIPDTYAEHLIAGGLMTQEEVSEIKSSYYAKLNDHLNNMAHYRPPALNLQA
HWQGLAQPEAQITTWSTGVPLDLLRFVGMKSVEVPRELQMHSHLLKTHVQVGSLQMAGYC
FSFLLSKGADVGLVFSVV

Homo sapiens Map View

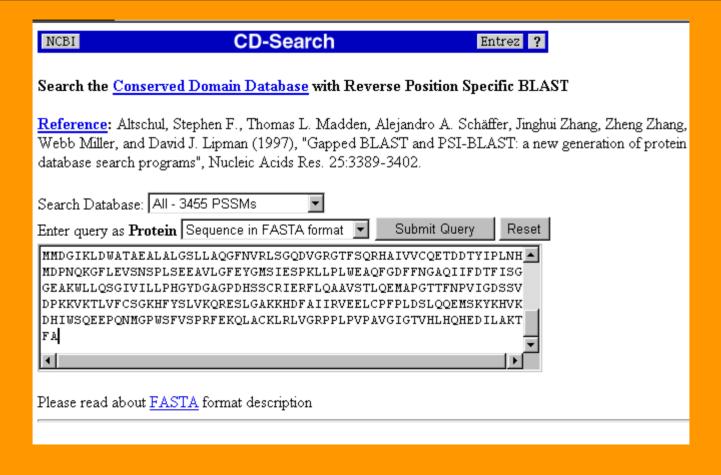
GenomeScan model Hs10_8856_22_1_2

CDS:

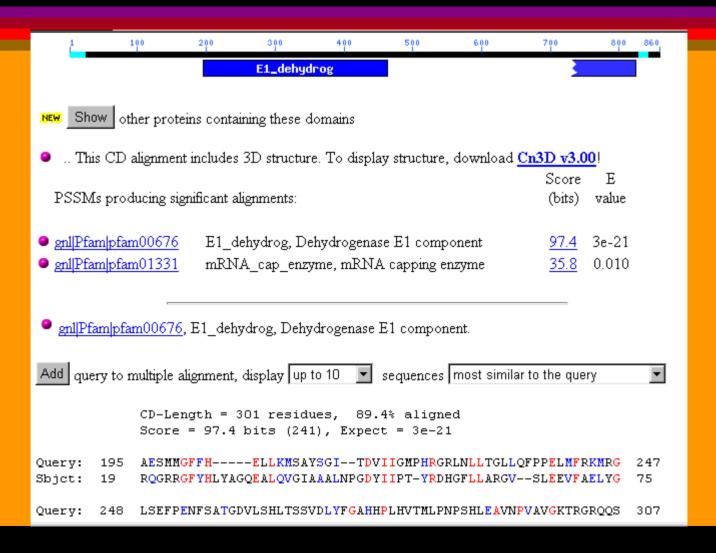
>1c1|Hs10 8856 22 1 2

MMDGIKLDWATAEALALGSLLAQGFNVRLSGQDVGRGTFSQRHAIVVCQETDDTYIPLNH
MDPNQKGFLEVSNSPLSEEAVLGFEYGMSIESPKLLPLWEAQFGDFFNGAQIIFDTFISG
GEAKWLLQSGIVILLPHGYDGAGPDHSSCRIERFLQAAVSTLQEMAPGTTFNPVIGDSSV
DPKKVKTLVFCSGKHFYSLVKQRESLGAKKHDFAIIRVEELCPFPLDSLQQEMSKYKHVK
DHIWSQEEPQNMGPWSFVSPRFEKQLACKLRLVGRPPLPVPAVGIGTVHLHQHEDILAKT
FA

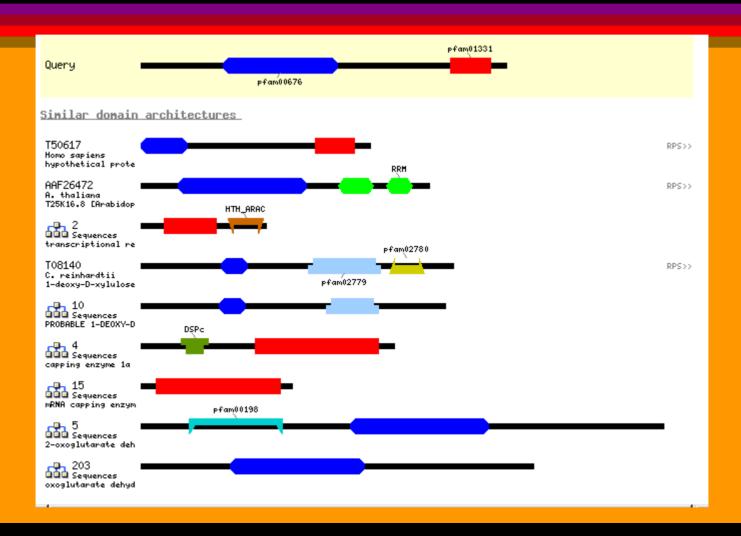
Do We Have a Whole Protein Domain



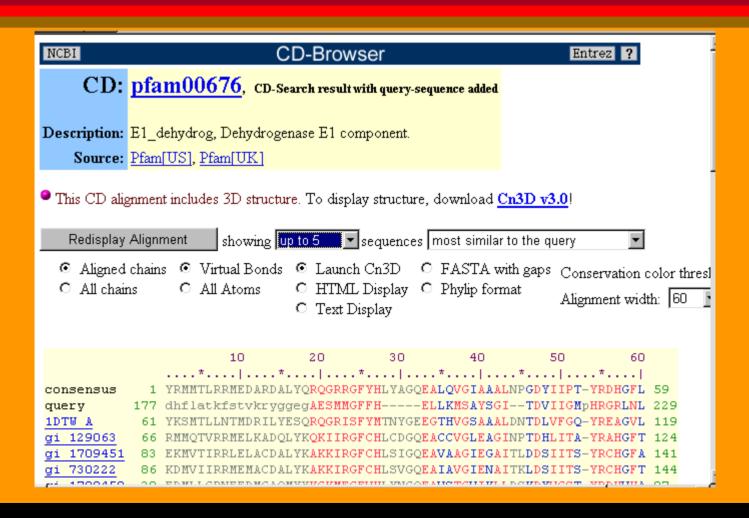
An E1 Dehydrogenase Domain



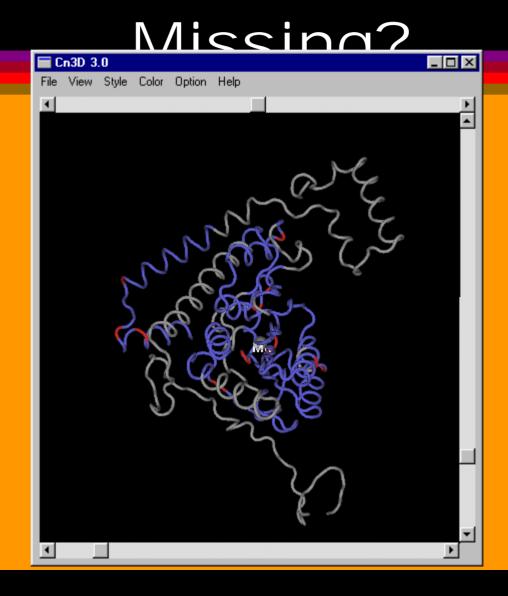
DART Results



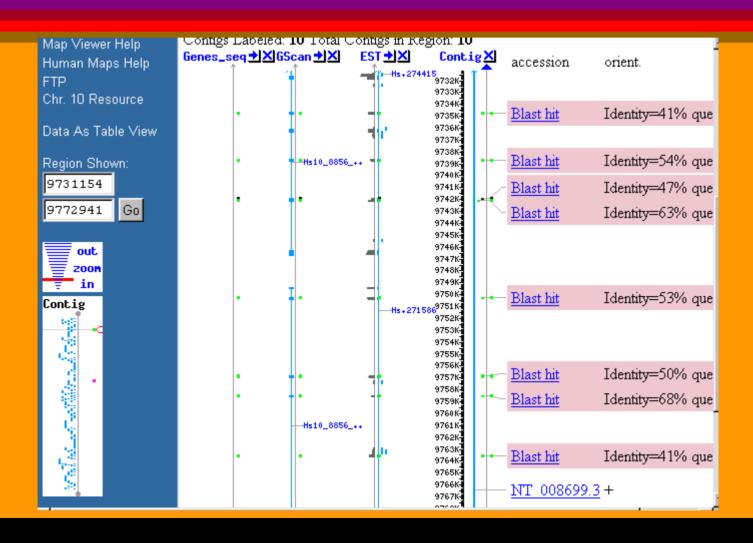
Visualizing the Domain HIt in 3D



Cn3D View of the Domain Hit: What is

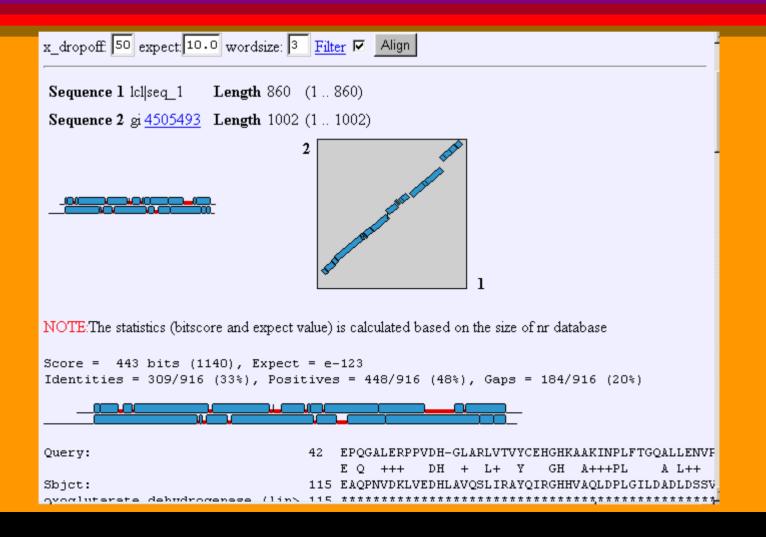


Did We Miss any Exons?



The Sequence of the Missing Exon

GenomeScan Protein vs the Probe



The Gap is Here

		580	${\tt AQGFNVRLSGQDVGRGTFSQRHAIVVCQETDD-TYIPLNHMDPNQKGFLEVSNSPLSEEA}$	638
			+G ++RLSGQDV RGTFS RH ++ Q D T IP+NH+ PNQ + V NS LSE	
		666	KEGIHIRLSGQDVERGTFSHRHHVLHDQNVDKRTCIPMNHLWPNQAPYT-VCNSSLSEYG	724
lrogenase	(lip>	666	********** ************	
		639	$\tt VLGFEYGMSIESPKLLPLWEAQFGDFFNGAQIIFDTFISGGEAKWLLQSGIVILLPHGYD$	698
			VLGFE G+ + SP L LWEAQFGDF N AQ I D FI G+AKW+ Q+GIV+LLPHG +	
		725	VLGFEAGLRMASPNALVLWEAQFGDFHNTAQCIIDQFICPGQAKWVRQNGIVLLLPHGME	784
lrogenase	(lip>	725	***************	
		699	GAGPDHSSCRIERFLQ	714
			G GP+HSS R ERFLQ	
		785	GMGPEHSSARPERFLQMCNDDPDVLPDLKEANFDINQLYDCNWVVVNCSTPGNFFHVLRR	844
lrogenase	(lip>	785	*****************	
_				
		715	GDSSVDPKKVKTLVFC	749
			A S+ EM PGT F VI G ++ +P+ VK L+FC	
		845	QILLPFRKPLIIFTPKSLLRHPEARSSFDEMLPGTHFQRVIPEDGPAAQNPENVKRLLFC	904
lrogenase	(lip>	845	*****************	
_				
		750	SGKHFYSLVKQRESLGAKKHDFAIIRVEELCPFPLDSLQQEMSKYKHVKDHIWSQEEPQN	809
			+GK +Y L ++R++ AI R+E+L PFP D L +E+ KY + + W QEE +N	
		905	TGKVYYDLTRERKARD-MVGQVAITRIEQLSPFPFDLLLKEVQKYPNA-ELAWCQEEHKN	962
lrogenase	(lip>	905	********* ***** ******************* ****	
_				
		810	MGPWSFVSPRFEKQLA 825	
			G + +V PR ++	
		963	QGYYDYVKPRLRTTIS 978	
lrogenase	(lip>		******	
_	_			

Finding a Structural Template for a Protein Sequence

Visualizing the Superposition of Sequence on Structure with Cn3D

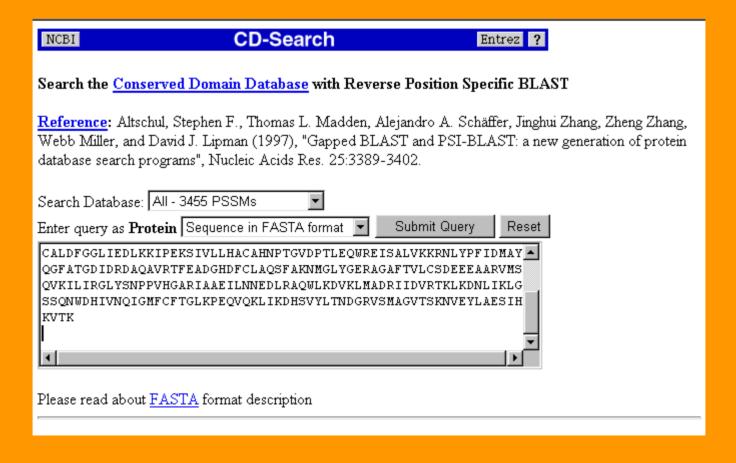
GenScan Predicted Peptide and ScanProsite Results

>14:10:32 GENSCAN_predicted_peptide_2 424_aa
MSQICKRGLLISNRLAPAALRCKSTWFSEVQMGPPDAILGVTEAFKKDTNPKKINLGAGA
YRDDNTQPFVLPSVREAEKRVVSRSLDKEYATIIGIPEFYNKAIELALGKGSKRLAAKHN
VTAQSISGTGALRIGAAFLAKFWQGNREIYIPSPSWGNHVAIFEHAGLPVNRYRYYDKDT
CALDFGGLIEDLKKIPEKSIVLLHACAHNPTGVDPTLEQWREISALVKKRNLYPFIDMAY
QGFATGDIDRDAQAVRTFEADGHDFCLAQSFAKNMGLYGERAGAFTVLCSDEEEAARVMS
QVKILIRGLYSNPPVHGARIAAEILNNEDLRAQWLKDVKLMADRIIDVRTKLKDNLIKLG
SSQNWDHIVNQIGMFCFTGLKPEQVQKLIKDHSVYLTNDGRVSMAGVTSKNVEYLAESIH
KVTK

[GS]-[LIVMFYTAC]-[GSTA]-K-x(2)-[GSALVN]-[LIVMFA]-x-[GNAR]-x-R-[LIVMA]-[GA] [K is the pyridoxal-P attachment site]

270-283 SFAKNMGLYGERAG

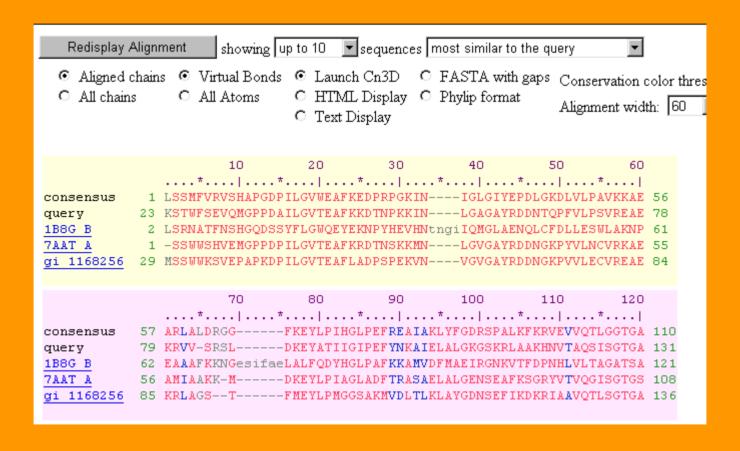
Begin with a CDD Search



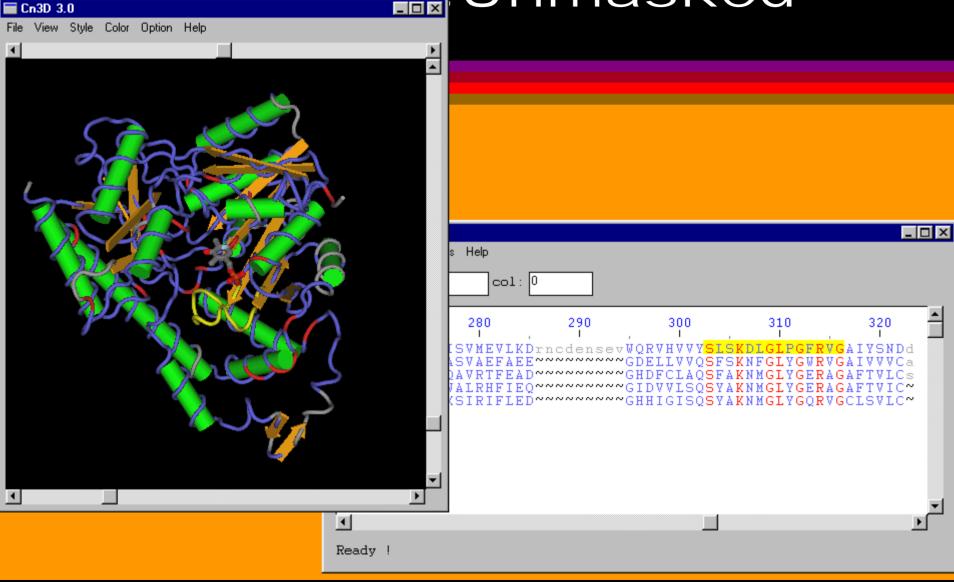
A Domain Hit...

```
gnl|Pfam|pfam00155, aminotran 1, Aminotransferase class-I.
Add query to multiple alignment, display up to 5 sequences most similar to the query
             CD-Length = 404 residues, 99.8% aligned
             Score = 410 \text{ bits } (1055). Expect = 5e-116
Query: 23
             KSTWFSEVOMGPPDAILGVTEAFKKDTNPKKINLGAGAYRDDNTOPFVLPSVREAEKRVV
Sbjct: 1
            LSSMEVRVSHAPGDPILGVMEAFKEDPRPGKINIGLGIYEPDLGKDLVLPAVKKAEARLA
Ouerv: 83
            -SRSLDKEYATIIGIPEFYNKAIELALGKGSKRLAAKHNVTAOSISGTGALRIGAAFLAK
                                                                           141
Sbjct: 61
            LDRGGFKEYLPIHGLPEFREAIAKLYFGDRSPALKFKRVEVVQTLGGTGALRLAADFLAN
                                                                           120
Ouerv: 142 FWOGNREIYIPSPSWGNHVAIFEHAGLPVNRYRYYDKDTCALDFGGLIEDLKKIPEKSIV
                                                                           201
Sbict:
       121 P---GDEVLVPDPTWPNYADIFKAAGFEVIPYRYYDENNFKLDFEALEAAITEAPEKTKV
                                                                           177
Querv:
       202 LLHACAHNPTGVDPTLEQWREISALVKKRNLYPFIDMAYQGFATGDIDRDAQAVRTFEAD
                                                                           261
Sbict:
       178 LLHNNPHNPTGTDPTREQLKKIAAVVKEKNILLLSDEAYQGFVFGDL--DAASVAEFAEE
                                                                           235
       262 GHDFCLAQSFAKNMGLYGERAGAFTVLCSDEE-----EAARVMSQVKILIRGLYSNPPV
Querv:
                                                                           315
Sbjct:
       236 GDELLVVQSFSKNFGLYGWRVGAIVVVCAIINAAAKKSSAGRVSSQLQSLARAMYSNPPD
                                                                           295
Ouerv:
       316 HGARIAAEILNNEDLRAQWLKDVKLMADRIIDVRTKLKDNLIKLGSSQNWDHIVNQIGMF
                                                                           375
Sbjct:
       296 HGAEIVARILSRPDLFTSWLEEVKGMACRIPNGRFYLWPDLSKLGRPE--DHIFEQDGMF
                                                                           353
Query: 376 CFTGLKPEQVQ-KLIKDHSVYLTNDGRVSMAGVTSKNVEYLAESIHKVTK 424
Sbjct: 354 SFTLLEEAOVVVIPGSEFGIYEPGWGRISLAGLSEANVDEAAERIRAFVK 403
```

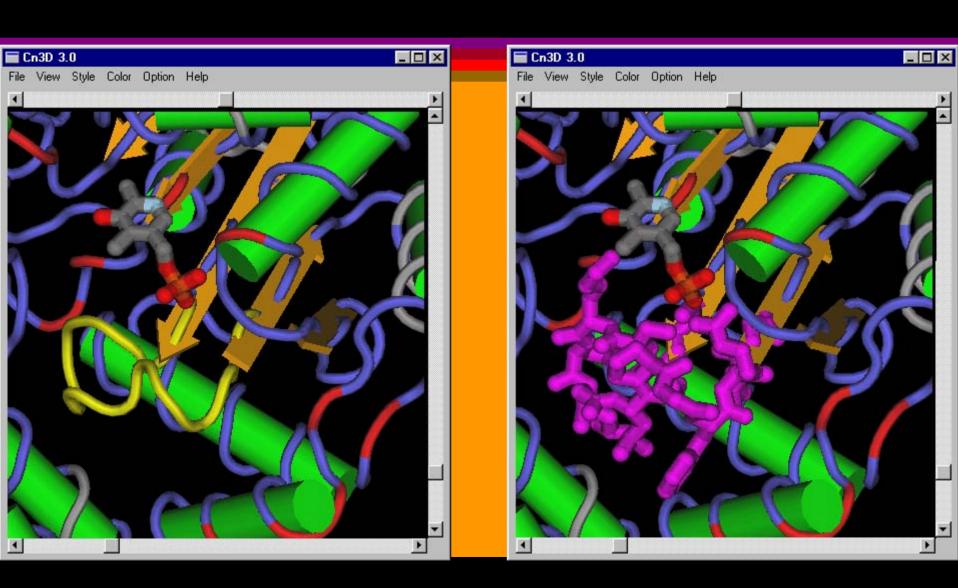
A Template Emerges...



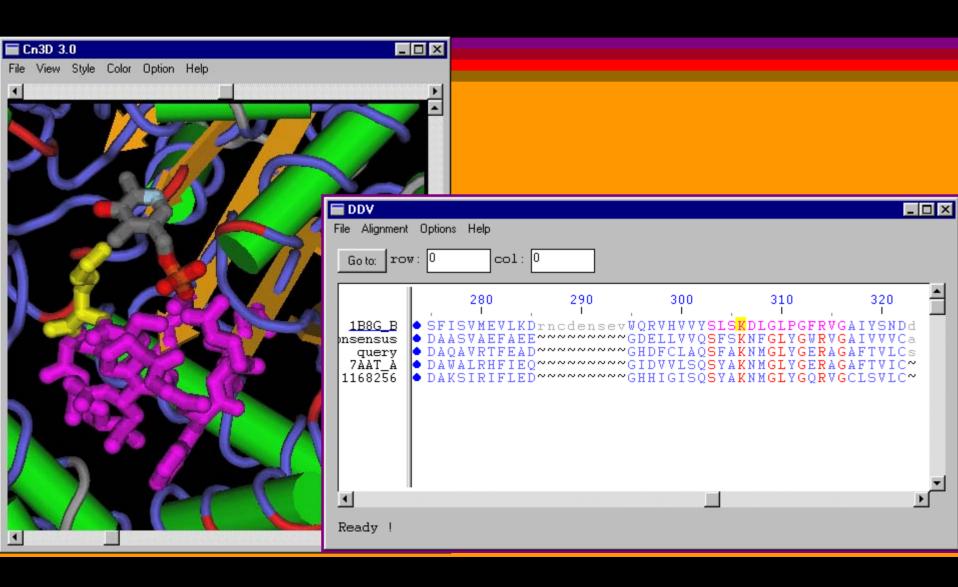
The Prosite Motif ——Unmasked



Close-ups of the Motif



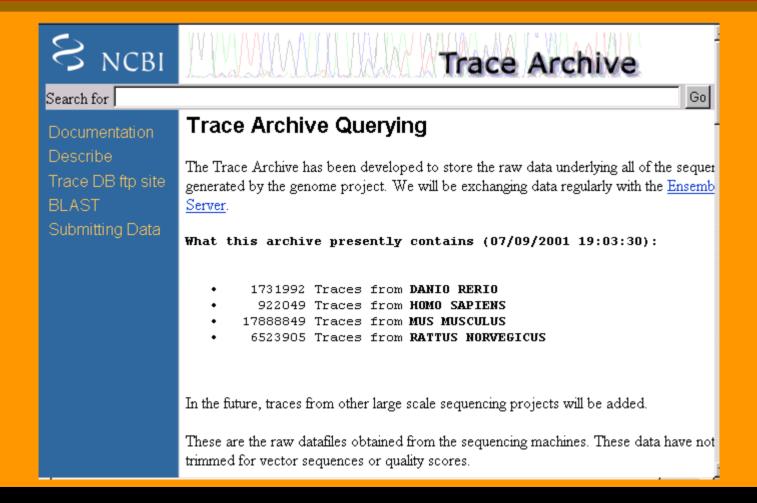
The Critical Lysine



Piecing Together a Gene from the Mouse Trace Archive

NX-57: A Mouse Kidney-Specific Membrand Protein

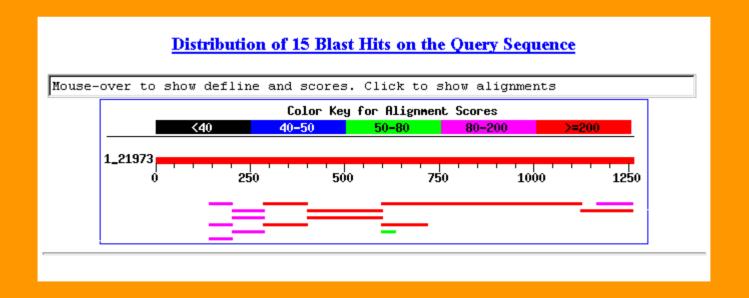
The NCBI Trace Archives



BLAST Against WGS Reads

S NCBI Nucleotide Pr	megablast BLAST otein Translations Retrieve results for an RID
Trace Archive database	Mega BLAST search
Search	MM_020526
Load query file from disk	Browse
<u>Set subsequence</u>	From: To:
<u>Database</u>	mmtrace 🔻
Return alignment endpoints only	

The Coverage is Good, but None of the Reads Completely Spans an Intron



Genomic Comparisons

COGs & Taxplot

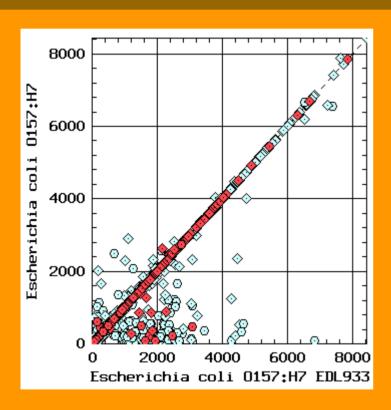
K12 vs O157 COGwise

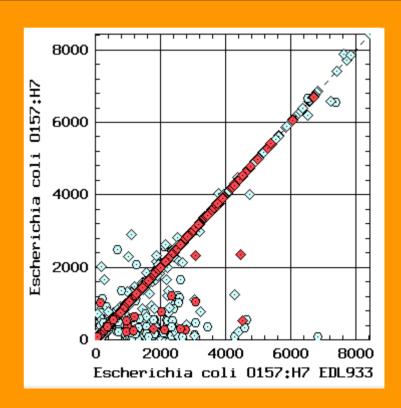
Escherichia coli k12	-	x	-	x	-	x	-	x	
Escherichia coli 0157	-	-	x	x	-	-	x	x	E - Enterobacteriaceae
Buchnera sp. APS	-	-	-	-	x	x	x	x	
Function	<u>1219</u>	<u>50</u>	<u>64</u>	<u>1281</u>	2	2	-	<u>548</u>	
JKL	<u>187</u>	9	9	<u>141</u>	-	-	-	<u>172</u>	Information storage and processing
J	<u>78</u>	-	-	<u>19</u>	-	-	-	<u>116</u>	Translation, ribosomal structure and biogenesis
K	<u>49</u>	<u>3</u>	4	<u>52</u>	-	-	-	<u>16</u>	Transcription
L	<u>60</u>	<u>6</u>	<u>5</u>	<u>70</u>	-	-	-	<u>40</u>	DNA replication, recombination and repair
DOMNPT	<u>161</u>	9	<u>11</u>	<u>329</u>	-	1	-	<u>129</u>	Cellular processes
D	<u>6</u>	-	-	<u>15</u>	-	-	-	<u>10</u>	Cell division and chromosome partitioning
0	<u>27</u>	-	<u>3</u>	<u>46</u>	-	-	-	<u>30</u>	Posttranslational modification, protein turnover, chaperones
М	<u>32</u>	<u>3</u>	1	<u>85</u>	-	-	-	<u>27</u>	Cell envelope biogenesis, outer membrane

Some Differences...

```
9 COGs
             Protein/Gene name:
                                                                      Select
                                                                               Help
                  D Cell division and chromosome partitioning
                  O Posttranslational modification, protein turnover, chaperones
                 M Cell envelope biogenesis, outer membrane
Functional categories:
                  N Cell motility and secretion
                  P Inorganic ion transport and metabolism
                  T Signal transduction mechanisms
Only Escherichia coli k12
                                               COG2747 Negative regulator of flagelli:
   7 -----qv---b-efq-----
                                         [ KN]
                                   Fl qM
                                               COG0562 UDP-galactopyranose mutase
   8 --m-----dr---e-----w
                                         \lceil \mathbf{M} \rceil
                                   G1 f
  28 a-mpkz---drlbcef-hsnuj----
                                  RfbC
                                         [M]
                                               COG1898 dTDP-4-dehydrorhamnose 3,5-epii
  26 aompkz-q-drlbcef--sn-jx---
                                               COG1091 dTDP-4-dehydrorhamnose reducta:
                                         [M]
                                  RfbD
   3 -----efq-----
                                               COG3190 Flagellar biogenesis protein
                                   Fli0
                                         [N]
                                               COG3031 General secretion pathway prote
   4 -----q----e-q------
                                         [N]
                                   GspC
                                               COG3156 General secretion pathway proto
   6 -----efq-s--j----
                                        [N]
                                   GspK
   3 ------
                                         [N]
                                               COG3149 General secretion pathway prote
                                   EtpM
   5 ----j----
                                         [N]
                                               COG3297 General secretory pathway prote
                                   GspL
```

Divergence from K12 Seen Using the TaxPlot

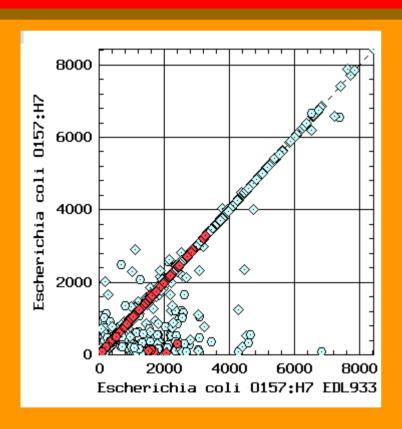


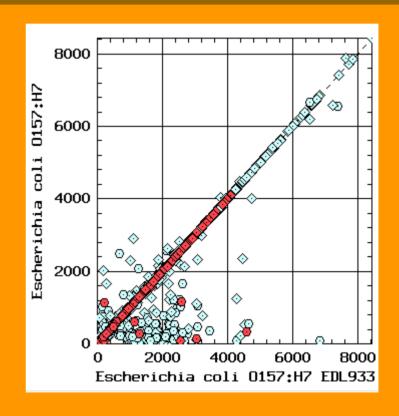


Amino Acid Transport & Metabolism

Energy Production

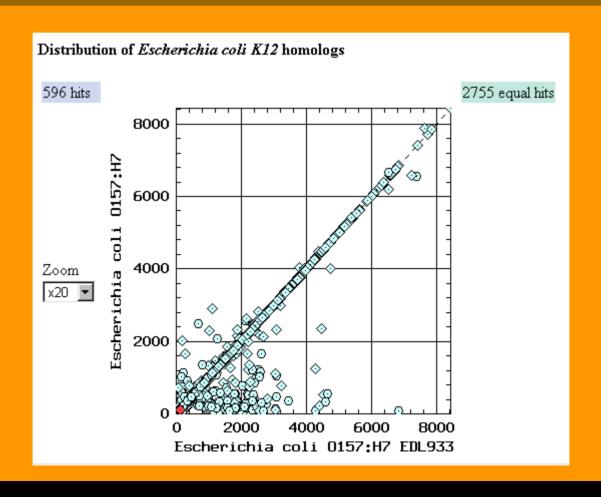
Divergence from K12





Inorganic Ion Transport

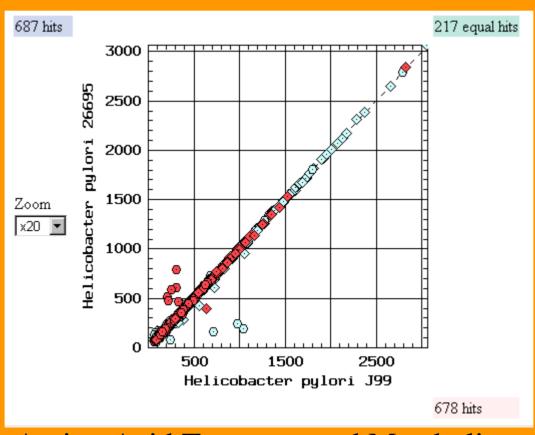
YadC: The Most Divergent Gene



The Two Pylori's COGwise

Helicobacter pylori 26695	-	x	-	x	-	x	-	x	
Helicobacter pylori J99	-	-	x	x	-	-	x	x	U - Proteobacteria epsilon
Campylobacter jejuni	-	-	-	-	x	x	x	x	subdivision
Function	2004	4	7	<u>100</u>	<u>258</u>	<u>3</u>	2	<u>788</u>	
JK L	<u>278</u>	1	-	<u>14</u>	<u>22</u>	-	-	<u>203</u>	Information storage and processing
J	<u>92</u>	-	-	1	<u>6</u>	-	-	<u>114</u>	Translation, ribosomal structure and biogenesis
K	<u>84</u>	-	-	<u>3</u>	<u>10</u>	-	-	<u>27</u>	Transcription
L	<u>102</u>	1	-	<u>10</u>	<u>6</u>	-	-	<u>62</u>	DNA replication, recombination and repair
DOMNPT	<u>328</u>	1	1	<u>25</u>	<u>62</u>	2	1	<u>220</u>	Cellular processes
D	<u>15</u>	-	-	4	-	-	-	<u>12</u>	Cell division and chromosome partitioning
0	<u>49</u>	-	-	<u>5</u>	<u>5</u>	-	-	<u>47</u>	Posttranslational modification, protein turnover, chaperones
М	65	_	_	6	14	2	_	61	Cell envelope biogenesis, outer membrane

K12 vs H. pylori Using the TaxPlot



Amino Acid Transport and Metabolism